

**INFLUENCE OF HYDROCORTISONE ON THE SUSCEPTIBILITY OF CHICKENS
TO THE NEMATODE, ASCARIDIA GALLI (SCHRANK, 1788)**

by

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B. S. Bethany College, Lindsborg, Kansas, 1960

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

**KANSAS STATE UNIVERSITY
Manhattan, Kansas**

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TABLE OF CONTENTS

INTRODUCTION	1
REVIEW OF LITERATURE	1
MATERIALS AND METHODS	12
EXPERIMENTAL RESULTS	16
Test I	16
Test II	19
Test III	20
Test IV	24
Test V	30
DISCUSSION	35
SUMMARY	38
ACKNOWLEDGMENT	40
REFERENCES	41

INTRODUCTION

The large intestinal roundworm, Ascaridia galli, is a common parasite of chickens. This nematode is said to be responsible for the "hidden" loss in domestic flocks, especially when it is present in the tissue phase of its life cycle. Young chickens are more susceptible than older birds to infections of this parasite. Some other factors that influence the susceptibility of the chicken to A. galli that have been investigated are vitamins, antibiotics, protein supplements in feed and the genetic make-up of the chicken.

Because there is a wide variation in worm burden within a group of birds receiving the same number of worm eggs, hormonal factors have been implicated in the natural resistance of the host to the parasite. The effect of gonadal and thyroid hormones on worm burden have been previously investigated. The purpose of this investigation was to test the effect of the adrenal-cortical hormone, hydrocortisone, on the worm burden of the chicken.

REVIEW OF LITERATURE

Among the various means by which an animal reacts to the invasion of foreign agents are inflammation of tissue and the development of an antibody/antigen reaction (immunological reaction). It has been shown that stress in vertebrates is followed by complex hormonal changes which are associated, among other things, with a decrease in resistance to bacterial infection. This decrease in resistance to infection is due in part to the production of adrenocorticotropic hormone (ACTH) from the pituitary gland and consequent release of adrenal glucocorticoids which, among other things, diminish normal inflammatory responses and antibody production. (Selye 1946a,b, 1950 and 1959)

Dougherty (1953) described the actions of adrenocorticosteroids in causing the involution of lymphatic organs at the cellular level. These actions were the budding of lymphocyte cytoplasm, pycnosis, karyorrhexis and inhibition of homoplastic and heteroplastic lymphocytopoiesis. There appeared to be a differential susceptibility of the cells. Immature lymphocytes were more resistant to lymphocytolysis and mature small lymphocytes were more susceptible to pycnosis and destruction. He described the effect of adrenocorticosteroids on immunization as occurring in two ways: (1) they enhance antibody release and (2) they tend to diminish synthesis of antibodies so that in time there is less reaction. He stated that adrenocortical hormones inhibited inflammation primarily by suppressing progressive cellular damage and therefore interrupted the chain reaction which follows an inflammatory stimulus.

Robinson and Smith (1953) reported that three factors of importance to the resistance of animals to infections appeared to be altered in adrenal-cortical hormone treated animals. These three factors were: (1) the inability to mobilize polymorphonuclear leucocytes at normal rate, (2) the failure of the reticuloendothelial system to remove and destroy foreign cells at a normal rate and (3) the suppression of the formation of antibodies.

The hypothesis that cortisone interferes with the functioning of the reticuloendothelial system, impairing its normal capacity to remove, fix or detoxify bacteria and certain products was formulated by Thomas (1953). The result of this interference may be a shifting of these functions to tissues not normally concerned with them, the outcome being uncontrolled infection or tissue damage.

A decided inhibition of antibody formation was found when cortisone or ACTH was administered during immunization (Fischel, 1953 and Bjørneboe,

Fischel and Stoerk, 1951). Earlier Fischel, Stoerk and Bjørneboe (1951) failed to show that cortisone had any effect on the rate of disappearance of circulating antibody protein.

Michael and Whorton (1951) presented evidence to show that the effect of cortisone on the delay of the inflammatory response could be observed from the moment of initiation of the inflammatory stimulus. The migration of leucocytes from blood vessels, formation of fibrin and edema were strikingly reduced and the ultimate development of necrosis was diminished. Dougherty, Brown and Berliner (1958) presented evidence which indicated that the presence of cortisol for a prolonged period within an area was not necessary to inhibit inflammation since cortisol reached minimal value in 100 minutes while inhibition of inflammation continued. They suggested that cortisol entered inflamed tissue in a nonspecific manner, produced its antiphlogistic effects and then was excreted. Since inflammation continued to be suppressed after the hormone concentration decreased, it is probable that cortisol produced some alteration within the tissue which resisted the inflammatory process. This alteration was said to be the binding up of the fibroblast. The rupture of this cell type is thought to be responsible for triggering the inflammation process.

The action of large dosages of cortisone or ACTH in increasing the susceptibility of a host to a parasite, as well as the role of these hormones in activating latent infection, has been described with regard to a wide variety of biological agents including bacteria, virus, protozoa and helminthes.

Robinson and Smith (1953) showed that large doses of ACTH, cortisone, and Compound F (hydrocortisone) lowered the resistance of white rabbits to a pneumococcus per se (rate of growth, virulence or morphology) and hence the deleterious effect of the hormone on the resistance to infections appeared to be

associated with altered host resistance.

Thomas (1952) reported production of increased susceptibility to lethal infections of microorganisms and increased susceptibility to necrolytic vascular damage by certain bacterial toxins in rabbits with injections of cortisone. Mogabgab and Thomas (1952) reported similar results, finding a decrease in lymphocyte count and suppression of inflammatory processes. Thomas (1953) found that the effects of cortisone on the response to bacterial infection and the action of gram negative bacterial toxins may be imitated by colloidal materials capable of "blockading" the reticuloendothelial system.

Cortisone enhanced the susceptibility to experimental poliomyelitis in hamsters according to Schwartzman and Aronson (1953). ACTH failed to modify the experimental infection. The effect of cortisone was in inhibiting the inflammatory response.

Kalter, Smolin, McElhaney and Tepperman (1951) treated mice with ACTH and cortisone and found a depression of multiplication of influenza virus. Kilborne and Horsfall (1951a), however, demonstrated an augmentation in the multiplication of influenza A and B types and mumps virus in chick embryos treated with cortisone. Kass, Ingbar, Lundgren and Finland (1951) found that cortisone increased the mortality of mice with influenza infection.

Kilborne and Horsfall (1951b) were also successful in obtaining a fatal Coxsackie disease in cortisone-treated adult mice.

An increased death rate and lesions were more widely spread following the use of cortisone on mice, guinea pigs and rabbits which had been infected with Brucella abortus, B. melitensis and B. suis. (Abernathy, 1951)

An attempt was made to modify susceptibility to Toxoplasma in chicks by Jones, Melton, Lunde, Eyles and Jacobs (1959) with the use of subcutaneous

Injection of cortisone. No symptoms were observed in these birds and they all survived as well as the controls.

Twenty-two of 59 cortisone-treated monkeys developed lesions of trypanosomiasis (*Trypanosoma cruzi*) in the nervous system, heart, fat bone marrow, striated muscle, lymph nodes and liver in work reported by Wolf, Kabat, Beiger and Fonseca (1953). The other treated animals had lesions free of parasites but resembling those of trypanosomiasis. The 20 control animals were all free of trypanosomal lesions. The authors concluded that cortisone enhanced the infection.

Redmond (1952) reported an enhancement of malarial infections in pigeons with cortisone treatment.

Berlin, Johnson, Hawk and Lawrence (1953) described a bacteremia and ultimate death of cortisone treated mice. The bacteremia consisted of organisms which are regarded as normal inhabitants of the gastrointestinal tract of the mouse. The authors emphasized the significance of the alteration of the host-parasite relationship which occurs in "normal" laboratory animals treated with large doses of cortisone. They reasoned that the lowering of host resistance was due possibly to an effect on the natural protective mechanisms.

Nobel (1961) subjected 226 squirrels to various stressors ie. light, heat, noxious stimulants, noise, crowding, darkness, hunger, annoyance, extreme confinement or caging. Controlled infections of cecal protozoa, mostly trichomonas, were administered to the squirrels. Over a period of two summers there was an average increase of 48 percent in numbers of protozoa in the stressed animals.

Adrenalectomy increased natural and acquired resistance of mice to infections with Trichinella spiralis in work reported by Baughn (1952).

That the reduction in worm burden was the result of reduction in the amount of glucocorticoids was shown by the fact that the administration of desoxy-corticosterone (DOC) to adrenalectomized mice did not alter the worm burden compared to other adrenalectomized animals. A reduction of an average of 28.9 worms was noted in adrenalectomized animals infected four days after adrenalectomy and 36 worms in those infected 11 days after adrenalectomy.

Stoner and Godwin (1953) measured the effects of ACTH and cortisone upon susceptibility to trichinosis in mice and found that neither favorably alter the course of experimental trichinosis in mice. ACTH increased susceptibility slightly while cortisone increased susceptibility 45 percent. These same workers (1954) found that cortisone treatment of mice immunized by active infection of T. spiralis and then challenged with Trichinella larvae produced a significant breakdown of their acquired immunity to reinfection. ACTH produced a slight but not significant increase in mortality.

More adult worms (T. spiralis) were found in cortisone-treated mice (125.0) than in controls (73.4) in work reported by Coker (1955). Larval count was also higher in treated animals. Coker stated that cortisone prevented the immunity reaction in the small intestine, especially during the time it would normally develop. There was also a suppression in the cellular response of the mice to the larvae. Coker (1956a,b,c) studied the diaphragm and some tongue tissue to describe this cellular response of the mouse to T. spiralis. Between 21 and 30 days there was a striking cellular response in all mice except those receiving cortisone. The inhibitory effects of cortisone were temporary and ceased to operate soon after hormone injections were discontinued. The inhibitory effects were such, however, as to render immunized mice essentially non-immune and seemed to prevent the gain of acquired

immunity which non-immunized mice ordinarily develop between 11 and 14 days following an infection. There was a direct relationship between suppression of the cellular response in the intestine and the persistence of large numbers of adult T. spiralis in the intestine.

Davis and Read (1958) demonstrated an effect of behavior on the susceptibility of mice to T. spiralis infections. Two groups of mice were used. One group was allowed to interact, fight and set up social rank. The other group was kept in separate cages and not allowed to interact. They found that the initial resistance of mice to the infection was reduced when the mice were allowed to interact in a group. This suppression of resistance was seen in increased adult and larval worm burdens in the grouped mice. The possible significance of this work was seen since crowding of mice produces hypertrophy of the adrenal glands (Davis and Christian, 1957) and injections of adrenocorticals generally decrease resistance to infection. Under certain conditions, animals could become more susceptible or less susceptible to infection depending upon the activity of the adrenal glands which is in part dependent upon their sociophysiological stature.

Weinstein (1955) reported an increase in the number of Nippostrongylus muris found in white rats following cortisone treatment. There was also an increase in the length of the worms as compared to the immune controls. A suppression of the inflammatory response, a reduction of antibody titer and the retardation of host growth with accompanying metabolic changes were said to be the cause of decreased resistance.

Cortisone injections did not completely destroy antibody production in mice with Trichinella infection in work reported by Markell and Lewis (1957). However infections were increased in cortisone-treated mice 10-fold and it

was suggested that cortisone masked the delayed tissue hypersensitivity that causes resistance to adult worms.

Lord (1958) found that ACTH therapy in trichinosis minimized weight loss, decreased mortality, prolonged survival time, slightly reduced eosinophilia and left the tissue response unaltered. In contrast, cortisone had no beneficial effect and in large dosages was quite toxic. Lord could not determine if cortisone affected the response of muscle tissue to infection. The evidence suggested that more larvae were able to penetrate the intestinal mucosa in those animals receiving cortisone. The result of finding different actions between ACTH and cortisone was surprising since with few exceptions either of the drugs was thought capable of replacing the other in therapy. Two explanations were offered: (1) the cortisone level was too high and quantitatively different results would be obtained from a reduced dosage and (2) ACTH in stimulating the adrenal cortex resulted in the secretion of mineralocorticoids which act in a different manner to cortisone.

Other studies have demonstrated the effect of cortisone on the reversal of the host's innate immunity to helminth infections. Roman (1956) showed that mice, normally resistant to Strongyloides ratti, would become infected if they were treated with cortisone.

Briggs (1959) demonstrated that cortisone caused a reversal in the virtually absolute resistance of the mature white rat to infections with Litomosoides carini, a normal parasite of the cotton rat. It was earlier shown that following introduction of the infective larvae into this abnormal host the white rat develops a powerful antibody. Cortisone treatment (30-50 mg./kg. of body weight) resulted in an almost complete suppression of the natural immunity.

The Chinese hamster was shown to have an innate resistance to I. spiralis by Ritterson (1959). The refractoriness of this host was most clearly expressed against the tissue phase of the parasite and seemed not to be related to "natural" or early antibody response. Treatment with cortisone reversed this innate resistance. Ritterson suggested that cortisone acted by prolonging structural integrity of injured cells. Adrenalectomy did not impair expression of the innate resistance.

Cross (1960) showed that injections of cortisone in the white rat resulted in normal development of Nematospirodes dubious, a normal parasite of the white mouse. In untreated rats, after the larvae penetrated the intestinal mucosa an intense inflammatory reaction around the larvae resulted in a connective tissue cyst from which the worms were unable to emerge and re-enter the lumen. The cortisone-treated rats harbored worms in the lumen similar to the mice.

Cortisone treatment substantially reduced guinea pig resistance to Nipponostringylus brasiliensis and thereby permitted the development of some adult worms in the intestine in work reported by Parker (1961). Skin sections of the larval inoculation sites showed that there was a delayed inflammatory response and nodular formation around the larvae in the lungs of the treated guinea pigs.

Coker (1957) found that the natural immunity of mice to Schistosoma mansoni, a fluke, was not weakened by cortisone. On the contrary, it seemed that immunity was enhanced as measured by the number of flukes recovered from the hepatic portal tracts of mice seven to eight weeks following infections. Weinman and Hunter (1959) reported the same results. It is likely that cellular defenses play little part in the phases of natural immunity against this

parasite.

Bezubik (1960) demonstrated that daily intramuscular injections of 0.5 mg. and 1.0 mg. of hydrocortisone sodium succinate to hamsters and guinea pigs did not render them susceptible to infection with larvae of the sheep and rabbit strains of Strongyloides papillulosus.

There is little work reported on the effect of glucocorticoids on parasitism in birds. Pullin (1955) observed that daily injections of 1.5 mg. cortisone per pound of body weight to turkey poult did not alter or modify the course of experimental enterohepatitis. McGregor (1954) had earlier shown that ACTH treatment had no effect.

The natural resistance of chickens to A. galli has been described in numerous reports. Ackert and Wilmoth (1934) reported that a strain of heavy White Minorcas was more resistant to the nematode than a lighter strain of the same breed. Ackert, Eisenbrandt, Wilmoth, Glading and Pratt (1935) reported that heavy breeds (Rhode Island Reds, White Plymouth Rocks and Barred Plymouth Rock breeds) had significantly fewer and smaller worms than did light breeds (White Leghorn and White Minorca breeds).

Age resistance of chickens to A. galli was reported by Herrick (1926). Later it was found that this increase with age in resistance correlated with an increase of intestinal goblet cell number by Ackert and Edgar (1940). Ackert, Edgar and Frick, (1939) and Frick and Ackert (1948) found that these goblet cells secreted a thermostable factor which inhibited growth of the nematode.

Dietary factors influencing resistance to A. galli have been extensively studied. Ackert (1942) reported that deficiency of Vitamins A and B complex resulted in an increase infection. Vitamin D did not retard worm

development but protected the fowl against the effects of parasitism. Riedel and Ackert (1950) reported feeding skim milk and supplement of 14.2 percent soybean oil meal resulted in increased resistance. Hansen, Norris and Ackert (1953) added aureomycin and Vitamin B₁₂ as a supplement to the feed of chickens and found evidence of an increased resistance. Sadun, Keith, Pankey and Totter (1950) described a lowered natural resistance accompanying a deficiency of dietary pteroylglutamic acid.

Sadun (1948a) injected testosterone propionate to males and estradiol benzoate to immature female chickens and found an increased resistance to infection of A. galli. In six experiments, 51 control birds retained between 2.02 and 4.02 times as many worms as the 51 treated birds. Heavy doses of the hormones had no effect. Sadun stated that the action of gonadal hormones on resistance may be related to the release of antibodies through the pituitary-adrenal cortex-lymphocytic chain of action.

The action of the synthetic female sex hormone, diethylstilbestrol, on the resistance of chickens to infection was tested by Ackert and Dewhirst (1950). The hormone was given by tri-weekly injections. The worm count following autopsy showed an average of 11.3 worms per experimental bird and 15.5 worms per control bird. Statistical analysis of the data indicated that the injections of this sex hormone increased the resistance of young female chickens to the nematode.

Injections of methyl testosterone was found to cause an initial increase and a later retardation of growth of A. galli within the chicken. Todd and Crowdus (1951) fed methyl testosterone at a rate of 20 mg. per kilogram of growing mash and obtained the same results. In the latter case there was no difference in the number of worms between the treated and untreated birds.

Both harbored an average of 3.5 worms. Worms from the treated birds averaged 23.43 mm. in length while worms from the untreated birds averaged only 13.8 mm. This difference was described as being highly significant.

Todd (1949) added thyroactive iodocasein (Protamine) and thiouracil to the basal diet to test the effect on natural resistance and growth of the parasite worm in New Hampshire broilers. There was no significant difference in the percentage of development of A. galli found in mildly hyperthyroid, mildly hypothyroid or normal hosts. Specimens of the worm attained significantly greater lengths in mildly hyperthyroid birds.

Sadun (1948b) demonstrated that even small single infections with A. galli rendered chickens highly resistant to a subsequent reinfection. Earlier, Eisenbrandt and Ackert (1940) could demonstrate no protection in six of nine groups of chickens following repeated injections of whole worm antigens. Sadun (1949) could not demonstrate reaction against the tissue antigens of the worms by the precipitin test but did find characteristic oral precipitates with antibodies reacting in vitro with the metabolic products of the living larvae. A relationship between resistance in chickens that received a single or multiple infection and the amount of reacting antibodies was found.

MATERIALS AND METHODS

One hundred eighty-seven male White Leghorn chicks and 50 male Delaware Hampshire chicks were used during the course of this investigation. One-day old chicks were obtained from a local commercial hatchery. They were raised in electric brooders, fed a commercial ration and watered ad libitum during the experimental period.

When the birds were fourteen days old they were banded, weighed and

separated into their respective experimental groups of approximately equal weights. In tests I, II and III about fifty birds were used. One half of the birds were put into the treated group and the others were used as controls in each of these experiments. Tests IV and V also utilized 50 birds but they were divided into four groups (10, 10, 15 and 15 birds).

On the fourteenth day each chick was infected with 100^+ A. galli eggs per os. The eggs were cultured using an adaptation of the methods of Hansen, Olson and Ackert (1954), Hansen, Terharr and Turner (1956) and Larson (1957). A group of A. galli was placed in a mortar and pressure was applied by the pedestal until the worms were thoroughly macerated. Artificial digestive juice (1.0 percent pepsin and 0.5 percent hydrochloric acid) was then poured on the macerated worms and the mixture was allowed to stand for four to six minutes. The mixture was then poured through an 80-mesh screen into a Petri dish. The screen retained the worm cuticula and other debris. Tap water was added to the Petri dish and after the eggs had settled to the bottom of the dish, the supernatant solution was withdrawn. Three to four additional washings with tap water removed the artificial digestive juice.

All egg cultures were incubated at 30° C. to 33° C. for 14 days. A drop of 1:1000 merthiolate solution was added to 10cc. water in each Petri dish culture to inhibit mold growth (Larson, 1957).

The chickens were infected by feeding the eggs to the birds with a calibrated micropipette. A variation of the egg administration technique of Hansen, et al. (1956) was used. All water was withdrawn from the Petri dish egg culture and 10 to 15 ml. of a 1.25 M. sucrose solution was poured into the dish. After the eggs had been scraped from the bottom of the dish with a rubber-tipped spatula, the sugar-egg suspension was poured into a small bottle.

A drop of the suspension was placed on a glass slide and the eggs were counted under a compound microscope. When it was necessary to dilute the suspension, additional 1.25 M. sugar solution was added and eggs in several drops of the new suspension were counted. The suspension was diluted until the micropipette would deliver 100 \pm 10 eggs when filled to the calibration point.

Intramuscular injections of hydrocortisone acetate (Hydrocortone, Merck, Sharp and Dohme) were begun on the day of infection. Birds in the treated group of Test I received 1.25 mg. of hydrocortisone acetate every three days. In Tests II and III, birds in the treated groups received 0.625 mg. of hydrocortisone acetate every three days. In Tests IV and V, in which the birds were divided into four groups, Group A (15 birds) received 0.625 mg. hydrocortisone acetate every three days for the first 20 days of the test period and 0.1 cc. distilled water for the final 10 days. Group B (15 birds) received 0.1 cc. distilled water for the first 20 days and 0.625 mg. of hydrocortisone acetate for the final 10 days of the test period. Group C (10 birds) received 0.625 mg. of hydrocortisone acetate every three days for the entire test period and were used as treated control birds. Group D (10 birds) served as an untreated control group. In all tests birds in the untreated control groups received intramuscular injections of 0.1 cc. of distilled water every three days. Prior to the start of the first test, greater amounts of hydrocortisone were tried to test their effect on the birds. It was found that dosages greater than 1.25 mg. every three days resulted in a high mortality rate of birds due to various metabolic disturbances associated with glucocorticoid injections. Birds receiving the smaller dosage (0.625 mg. every three days) gained more weight.

Following treatment, birds were weighed and the weights were recorded every third day. Throughout the experimental period the birds were kept in

cages so that they would not be crowded.

The test period for Test I was 40 days. For the other tests the period was shortened to 30 days. On the final day of the test period the birds were weighed and sacrificed. Worms were recovered from the lumen of the small intestine of control birds by the hydraulic method of Ackert and Nolf (1929). The intestine from the gizzard to the yolk sac diverticulum was removed from the body cavity and was attached to a small water hose and the contents flushed into a 1000 ml. beaker. The flushings were poured through a 20-mesh screen which held back the worms. The worms were counted and preserved in 10 percent formalin solution. The adrenal glands of the chickens were removed and fixed in Bouins fixative for later study.

Worms were recovered from the treated birds by removing the small intestine from the gizzard to the yolk sac diverticulum and placing it in a flat pan of water. The small intestine was then slit manually with a pair of scissors and the contents of the intestine washed in the pan of water. The water and contents were poured through the 20-mesh screen and the worms recovered, counted and preserved as above.

In the event that worms would become fractionated, pieces were matched under the microscope as completely as possible according to sex and body size.

The worms recovered from Tests IV and V were measured. The image of each worm was projected through a lens in a photographic bellows which magnified the image of the worm six times. Each image was traced on tissue paper and measured with a Dietzgen planimeter.

Statistical analyses of the results were made by the Kansas State University Department of Statistics.

EXPERIMENTAL RESULTS

Test I

The effect of 1.25 mg. of hydrocortisone acetate injections on the worm burden of white Leghorn cockerels was observed in Test I. Thirty-seven birds were used; 19 birds were put into the treated group and 18 birds were used for controls. The experimental design of this test is shown in Table I.

Table I. Test I experimental design.

Age of birds (days)	:	14	:	14	-	54	:	54
Procedure	:	Infection of birds with <u>A. galli</u>	:	Treatment with 1.25 mg. hydrocortisone every three days	:	Sacrifice at 40 days post-infection		
Group:								
A (19 birds treated)		+		+		+		+
B (18 birds controls)		+		-		+		+

The 19 birds of Group A were given the injections of hydrocortisone acetate every three days for the entire test period. Each bird received a total of 17.5 mg. of hydrocortisone. Control birds received injections of distilled water. Three birds of the treated group (A) died during the test period from unknown causes.

All of the treated birds were found to harbor A. galli in their small intestines following sacrifice. A total of 521 worms was recovered from the treated group. Worm burden ranged in number from 18 to 81 worms per bird.

and the mean was 38.81 worms per hydrocortisone-treated bird. Fifteen of the control birds harbored worms, while the three others showed no sign of infection. The mean number of worms harbored by control birds was 7.83 worms with a range of 0 to 35 worms per bird. Analysis of variation in worm numbers between the groups gave an F value of 86.51 which is significant at the .001 level.

Hydrocortisone-treated birds gained less weight than control birds during the test. Treated birds gained an average of 34.55 grams and the control birds gained an average of 552.22 grams during the test period. Statistical analysis of variation in average weight gain between the treated and control groups gave an F value of 698.07 which is significant at the .001 level.

Experimental results of Test I are shown in Table 2 and worm burden distribution is shown in Figure 1.

Table 2. Effects of injections of 1.25 mg. hydrocortisone every three days on weight gain and worm burden of Leghorn cockerels.

Group	: Average weight : gain (gms)	: Average dally weight : gain (gms)	: Total number of worms : recovered	: Average number of worms per bird
A (treated)	34.55*	0.84	521	38.81*
B (controls)	552.22	13.81	141	7.83

*Significant at the .001 level

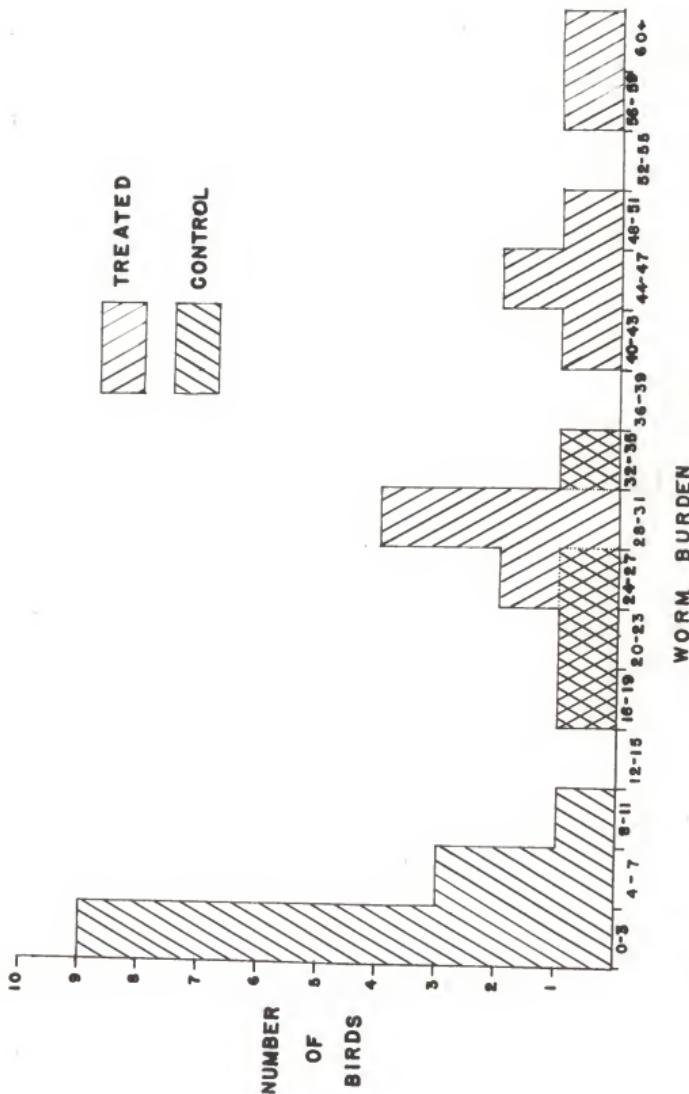


Figure 1. Distribution of worm burden of treated (125 mg. hydrocortisone/3 days/40 days) and control Leghorn cockerels.

Test II

In this test 0.625 mg. of hydrocortisone acetate injections were given to White Leghorn cockerels to observe the effect on worm burden and weight gain. Twenty-five birds were placed in both the experimental and control groups. Table 3 presents the experimental design for this test.

Table 3. Tests II and III experimental design.

Age of birds (days) :	14	:	14 - 44	:	44
Procedure	: Infection of : birds with : <u>A. galli</u>	: Treatment with 0.625 mg. : hydrocortisone acetate : every three days	: Sacrifice at : 30 days post- : infection		
Group:					
A (25 birds treated)	+	+	+		+
B (25 birds controls)	+	-		+	

Treated birds received a total of 6.25 mg. of hydrocortisone during the test period. All birds survived. Control birds were given injections of distilled water every three days.

Table 4 shows the experimental results of this test. Hydrocortisone-treated birds gained less weight than the control birds. The average weight gain for the treated birds was 54.84 grams and for the control birds the average was 350.16 grams for the test period. Statistical analysis of variation in the average weight gain between the treated and control groups gave an F value of 720.46 which is significant at the .001 level.

A total of 930 worms was recovered from the hydrocortisone-treated birds. All of the treated birds were found to harbor from 21 to 73 worms per bird. The mean number of worms per treated bird was 37.20. Worm burden of control birds ranged from 0 to 27. Then mean worm burden for the control group was 13.12 worms per bird. Statistical analysis of variation in worm numbers in the treated group and control group gave an F value of 65.66 which is significant at the .001 level. Worm burden distribution for Test II is shown in Figure 2.

Table 4. Effect of injections of 0.625 mg. hydrocortisone every three days on weight gain and worm burden of Leghorn cockerels

Group	: Average : weight : gain (gms)	: Average : daily weight : gain (gms)	: Total number : of worms : recovered	: Average : number of : worms per bird
A (treated)	54.84*	1.802	930	37.20*
B (controls)	350.16	11.75	338	13.12

*Significant at the .001 level.

Test III

The effect of 0.625 mg. injections of hydrocortisone acetate every three days on the susceptibility of a heavy breed chicken (Delaware Hampshire) to A. galli infection was observed in Test III. Twenty-five birds were placed in both the treated and control groups. The experimental design for this test is similar to Test II and is shown in Table 3.

As in the previous tests, the treated birds gained less weight than the control birds. The average weight gain for treated birds was 137.37 grams

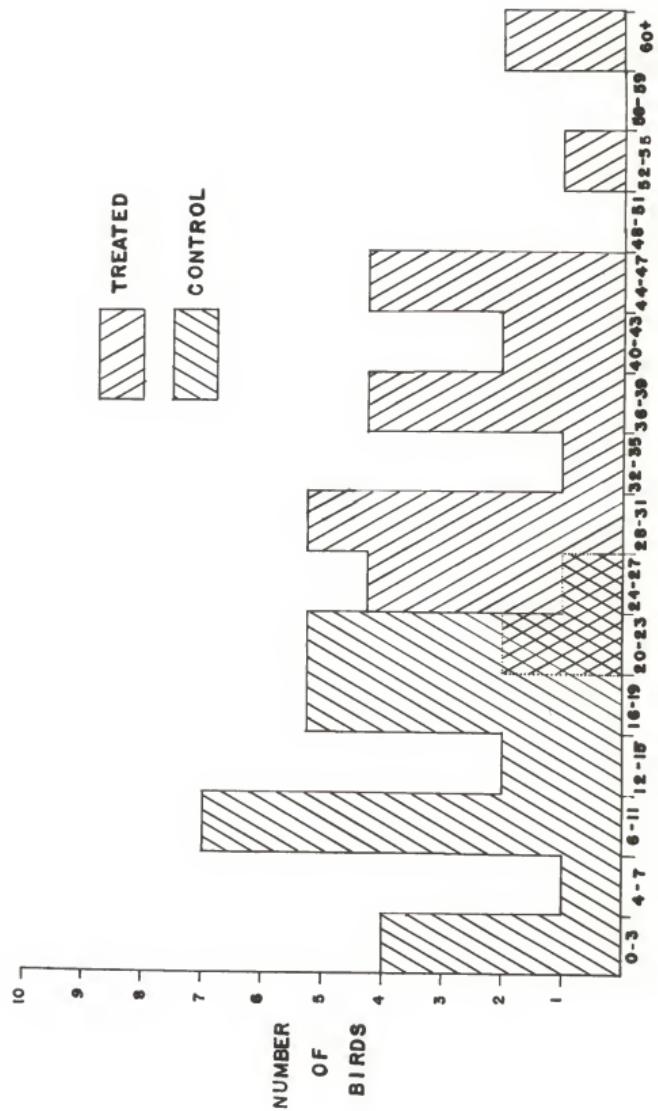


Figure 2. Distribution of worm burden of treated (0.625 mg. hydrocortisone/3 day/30 days) and control Leghorn cockerels.

and for the control birds 707.19 grams during the test period. The average daily weight gain for the treated birds was 4.65 grams and for the treated birds the average daily weight gain was 23.15 grams. Analysis of variance between the groups gave an F value of 698.07 which is significant at the .001 level. Table 5 shows the experimental results of this test.

Table 5. Effect of injections of 0.625 mg. hydrocortisone every three days on worm burden and weight gain of Delaware-Hampshire cockerels.

Group	: Average weight : gain (gms)	: Average daily weight : gain (gms)	: Total number of worms : recovered	: Average number of worms per bird
A (treated)	137.37*	4.65	562	22.48*
B (controls)	707.19	23.15	34	1.55

*Significant at the .001 level

All birds of the treated group were found to have A. galli in their small intestine. A total of 562 worms was recovered from the treated group. Worm burden of the treated group ranged from 7 to 53 worms per bird, the mean worm burden being 22.48. Thirteen of the control birds contained no worms. Thirty-four worms were recovered from the other birds in the control group. The mean number of worms per bird in the control group was 1.55 and the worm burden ranged from 0 to 12 worms per bird. Analysis of variance between the worm burden of treated and control birds gave an F value of 86.51 which is significant at the .001 level. The worm burden distribution for Test III is shown in Figure 3.

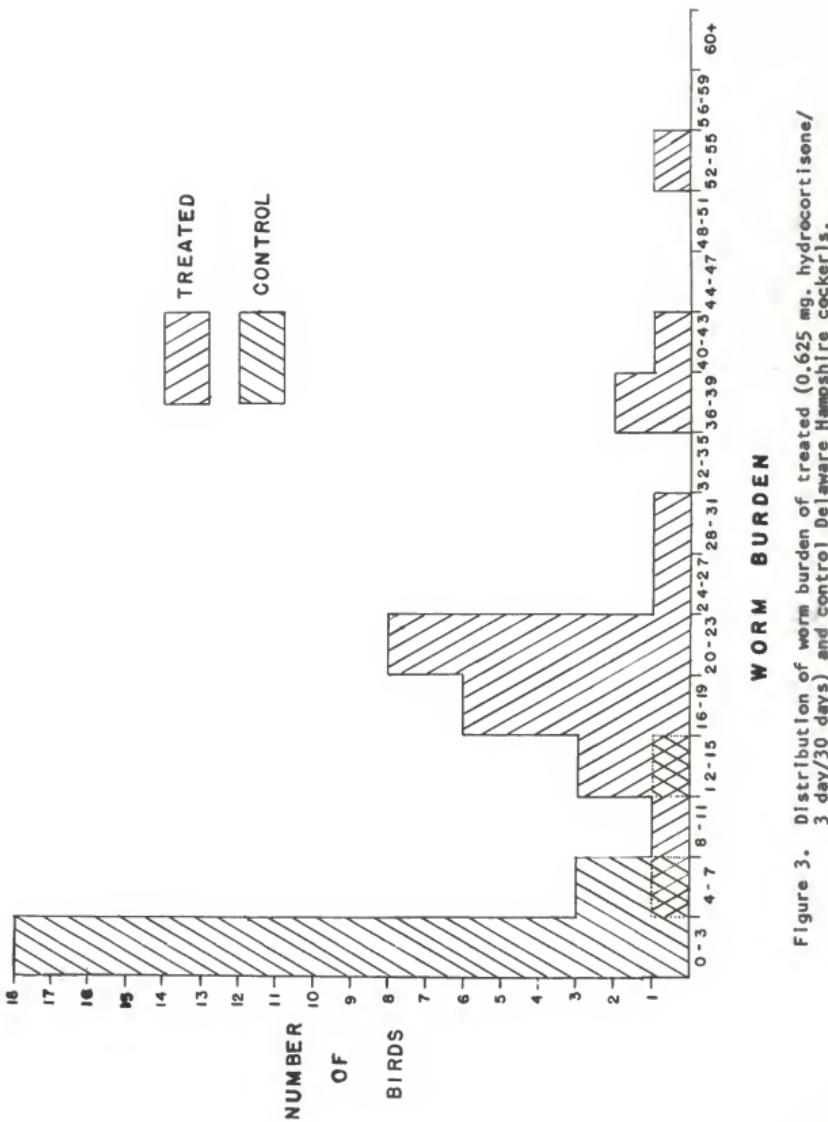


Figure 3. Distribution of worm burden of treated (0.625 mg. hydrocortisone/3 day/30 days) and control Delaware Hampshire cockerels.

Test IV

Test IV was designed to investigate the period that hydrocortisone was instrumental in increasing the worm burden of White Leghorn cockerels. The birds were divided into four groups. One group served as treated controls receiving hydrocortisone throughout the test period. A group of untreated birds was also kept for controls.

One group (Group A) of birds was given 0.625 mg. hydrocortisone injections every three days during the first 20 days post-infection and received a total of 4.325 mg. of hydrocortisone. A second group (Group B) of birds received a total of 1.875 mg. of hydrocortisone in 0.625 mg. injections every three days during the final 10 days of the 30-day test period. The treated control birds (Group C) received 0.625 mg. hydrocortisone injections every three days during the entire test period. Untreated control birds received injections of distilled water during the test period. Table 6 shows the experimental design for this test.

All treated birds gained less weight than the untreated control birds. The average weight gains for the test period were: Group A, 134.6 grams; Group B, 379.53 grams, Group C (treated controls), 75.40 grams, and Group D (untreated controls), 542.75 grams. During the period of hydrocortisone treatment, Group A birds gained an average of 1.31 grams a day. Following hormone treatment these birds gained an average of 10.85 grams a day. Prior to hydrocortisone treatment the birds in Group B gained an average of 13.75 grams per day while during treatment the average daily weight gain was 10.45 grams. Treated control birds had an average daily weight gain of 2.51 grams. Untreated control birds averaged 18.90 grams in weight gain per day. The effect of hydrocortisone treatment on weight gain for Test IV is shown in

Table 6. Test IV and V experimental design.

Age of birds days)	14	14---34	34---44	44
Procedure	Infection of birds with <i>A. galli</i>	Treatment with 0.625 mg. hydrocorilone acetate every three days	Sacrifice at 30 days post-infection	
A (fifteen birds)	+	+	-	+
B (fifteen birds)	+	-	+	+
C (treated controls)	+	+	-	+
D (untreated controls)	+	-	-	+

Table 7. Analysis of variation in average weight gain gave an F value of 401.89 which is significant at the .001 level.

Treatment with hydrocortisone during the first 20 days after infection increased the worm burden in the chickens to approximately the same level as did treatment throughout the test period. Treatment after 20 days post-infection did not increase worm burden. Group A had a mean worm burden of 34.87 with a range of 22 to 50 worms per bird. Worm burden in Group B ranged from 0 to 20 with a mean worm burden of 5.60 worms per bird. Treated control birds had a mean worm burden of 36.80 with a range from 18 to 73 worms per bird. The range of worm burden in untreated control birds was 0 to 12 with a mean worm burden of 3.60. Analysis of variation in worm number between treatment groups gave an F value of 57.60. Worm burden of Group A and the treated control group was significantly greater than the untreated control at the .001 level. Group B did not have a significantly greater worm burden than the untreated control birds. The effect of the period of hydrocortisone treatment on the worm burden is shown in Table 8. Worm burden distribution for Test IV is shown in Figure 4.

Statistical analysis indicated that male worms were longer and female worms were shorter in Group A the treated control birds than those worms from Group B and the untreated control birds. Tables 9 and 10 present the statistical data on the length of worms.

Table 7. The effect of 0.625 mg. hydrocortisone injections every three days on the weight gain
in grams of Leghorn cockerels.

Treatment	: Average weight gain first 20 days	: Average daily weight gain : first 20 days	: Average weight gain last 10 days	: Average daily weight gain : first 20 days	: Average weight gain for entire test period	: Average daily weight gain for test period
Group A 0.625 mg./3 days/ 20 days 0.1 cc. distilled water/3 days/ 10 days	26.13	1.307	108.47	10.85	134.60*	4.49
Group B 0.1 cc. distilled water/3 days/ 10 days 0.625 mg./3 days/ 10 days	275.00	13.75	104.53	10.45	379.53*	12.65
Group C treated controls 0.625 mg./3 days/ 30 days	35.70	1.785	39.70	3.97	75.40*	2.51
Group D untreated controls 0.1 cc. distilled water/3 days/ 10 days.	302.00	15.10	240.50	24.05	542.75	18.09

* Significant at the .001 level

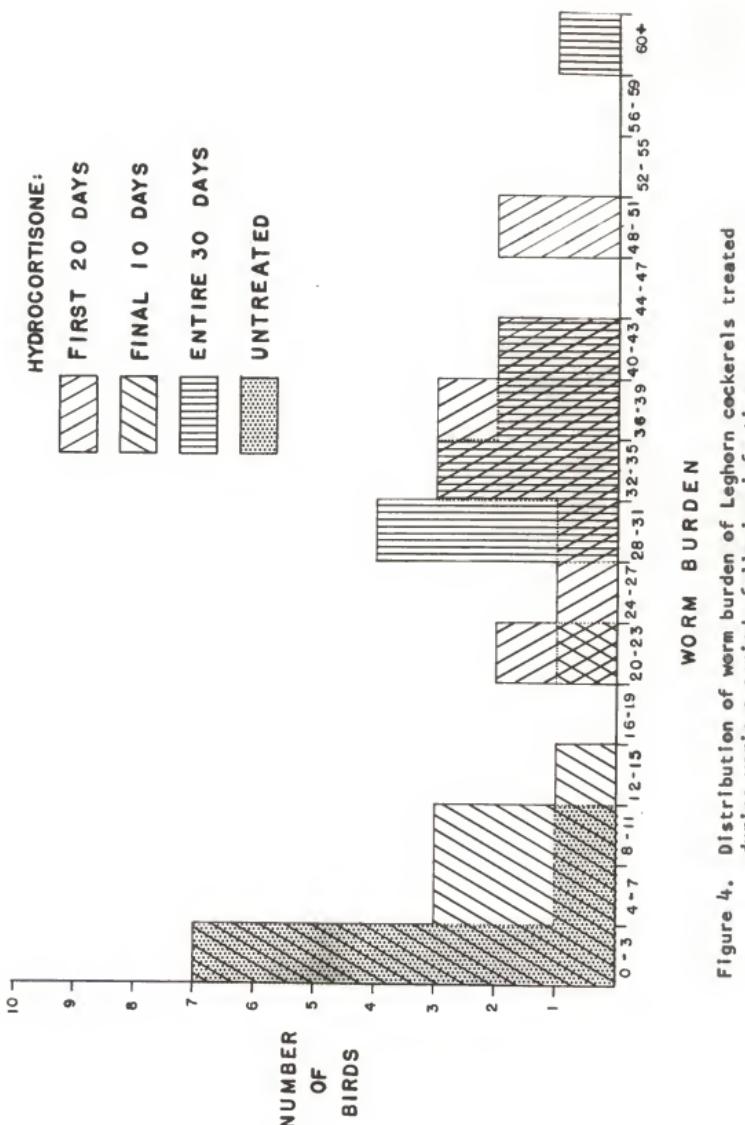


Figure 4. Distribution of worm burden of Leghorn cockerels treated during various periods following infection.

Table 8. Effect of period of hydrocortisone treatment on the worm burden of White Leghorn cockerels

Procedure and Treatment	:	Total worms recovered	:	Average number of worms per bird
Group A (15 birds) hydrocortisone treatment first 20 days post-infection.		523		34.87*
Group B (15 birds) hydrocortisone treatment from 20 to 30 days post-infection		84		5.60
Group C (10 birds) treated controls hydrocortisone treatment for entire 30 day test period		368		36.80*
Group D (10 birds) untreated controls		36		3.60

* Significant at the .001 level

Table 9. Statistical comparison of length of male worms of the different treated groups of Test IV.

Group	:	N	:	S	:	Ss	:	s	:	\bar{s}	:	\bar{x}
A (hydrocortisone first 20 days post-infection)		171		4145.0		2788.67		4.05		.31		4.04
B (hydrocortisone from 20 to 30 days post-infection)		25		614.5		469.34		4.42		.88		4.10
C (treated controls)		98		2303.0		1336.50		3.71		.37		3.92
D (untreated controls)		13		285.5		337.31		5.30		1.47		3.32

Legend: N = number of worms measured
 S = sum of the lengths of the worms
 Ss = sum of squares of deviations
 \bar{s} = $\sqrt{\text{sum of squared deviations/observations minus one}}$
 s = standard error of mean
 \bar{x} = mean length in centimeters

Table 10. Statistical comparison of length of female worms of the different treated groups of Test IV

Group	,	N	:	S	:	Ss	:	s	:	$s\bar{x}$:	\bar{x}
A (hydrocortisone first 20 days post-infection)	,	254	:	8394.0	:	4036.93	:	3.99	:	.25	:	5.51
B (hydrocortisone from 20 to 30 days post- infection)	,	49	:	1633.5	:	899.69	:	4.33	:	.62	:	5.56
C (treated controls)	,	208	:	6417.0	:	2456.38	:	3.44	:	.24	:	5.14
D (untreated controls)	,	23	:	766.5	:	216.80	:	3.14	:	.65	:	5.56

Legend: N = number of worms measured
 S = sum of the length of the worms
 Ss = sum of squares of deviations
 s = $\sqrt{\text{sum of square deviations/observations minus one}}$
 $s\bar{x}$ = standard error of mean
 \bar{x} = mean length in centimeters

Test V

Test V is similar to Test IV in design. Additional data was obtained on the period of hydrocortisone effectiveness in increasing worm burden. The experimental design is shown in Table 6.

During hydrocortisone treatment, birds in all groups gained less weight than untreated control birds. Group A birds gained an average of 134.40 grams, Group B gained an average of 256.07 grams, the treated control group gained an average of 78.60 grams and the untreated control group gained an average of 346.7 grams. Statistical analysis of variance of the weight gains

Table 11. The effect of 0.625 mg. hydrocortisone injections every three days on the weight gain in grams of Leghorn cockerels.

Treatment	Average weight : gain first 20 days	Average daily : weight gain : first 20 days	Average weight : gain last 10 days	Average daily : weight gain : last 10 days	Average weight : gain for entire : test period	Average daily : weight gain : for test period
Group A						
0.625 mg./ 3 days/ 20 days	33.80	1.69	100.60	10.60	134.40*	4.48
0.1 cc. distilled water/ 3 days/ 10 days						
Group B						
0.1 cc. distilled water/ 3 days/ 20 days	199.98	9.95	57.09	5.71	256.07*	8.54
0.625 mg./ 3 days/ 10 days						
Group C						
treated controls	47.80	2.39	30.80	3.08	78.60*	2.82
0.625 mg./ 3 days/ 30 days						
Group D						
untreated controls	182.79	9.14	163.90	16.39	346.70	11.56
0.1 cc. distilled water/ 3 days/ 30 days						

* Significant at the .001 level

of the various groups gave an F value of 95.23 which is significant at the .001 level. Weight gains of the various groups are shown in Table II.

The effect of the period of hydrocortisone injections on worm burden is shown in Table I2. The worm burden of Group A and that of the treated control birds were approximately the same. According to the results of this and the previous test, the effect of hydrocortisone in increasing worm burden is greater if given during the first 20 days following infection. No significant increase was found in the worm burden of birds treated with hydrocortisone after 20 days post-infection. Analysis of variation in worm numbers between treatment groups gave an F value of 101.95. The increase in worm burden of Group A and the treated controls is significant at the .001 level.

Table I2. Effect of period of hydrocortisone treatment on the worm burden of White Leghorn cockerels

Procedure and Treatment	: Total worms recovered	: Average number of worms per bird
Group A (15 birds) hydrocortisone treat- ment first 20 days post-infection	469	31.27*
Group B (14 birds) hydrocortisone treat- ment from 20 to 30 days post-infection	45	3.21
Group C (10 birds) treated controls hydrocortisone treat- ment throughout test period	326	32.60*
Group D (10 birds) untreated controls	37	3.70

* Significant at the .001 level

Worm burden of Group A ranged from 18 to 81 worms per bird with a mean of 31.27. Group B had a mean worm burden of 3.21 with a range from 0 to 10 worms per bird. Group C (treated controls) birds had a mean worm burden of 32.60 with a range of 23 to 51 worms per bird. The untreated controls had a mean worm burden of 3.70. Worm burden in the untreated controls ranged from 0 to 13. The worm burden distribution of Test V is shown in Figure 5.

As in Test IV statistical study of the standard deviation of worm length showed that male worms were longer and female worms were shorter in birds of Group A and the treated control group than those worms from the other two groups. Statistical data on the worm length is presented in Tables 13 and 14.

Table 13. Statistical comparison of length of male worms in the different treated groups of Test V.

Group	:	N	:	S	:	S _s	:	s	:	S _m	:	\bar{x}
A (hydrocortisone first 20 days post-infection)	:	153	:	3762.5	:	2232.72	:	3.83	:	.31	:	4.10
B (hydrocortisone from 20 to 30 days post-in- fection)	:	15	:	332.5	:	205.83	:	3.83	:	.99	:	3.70
C (treated controls)	:	104	:	2508.5	:	1763.25	:	4.14	:	.41	:	4.02
D (untreated controls)	:	14	:	289.5	:	243.30	:	4.33	:	1.16	:	3.45

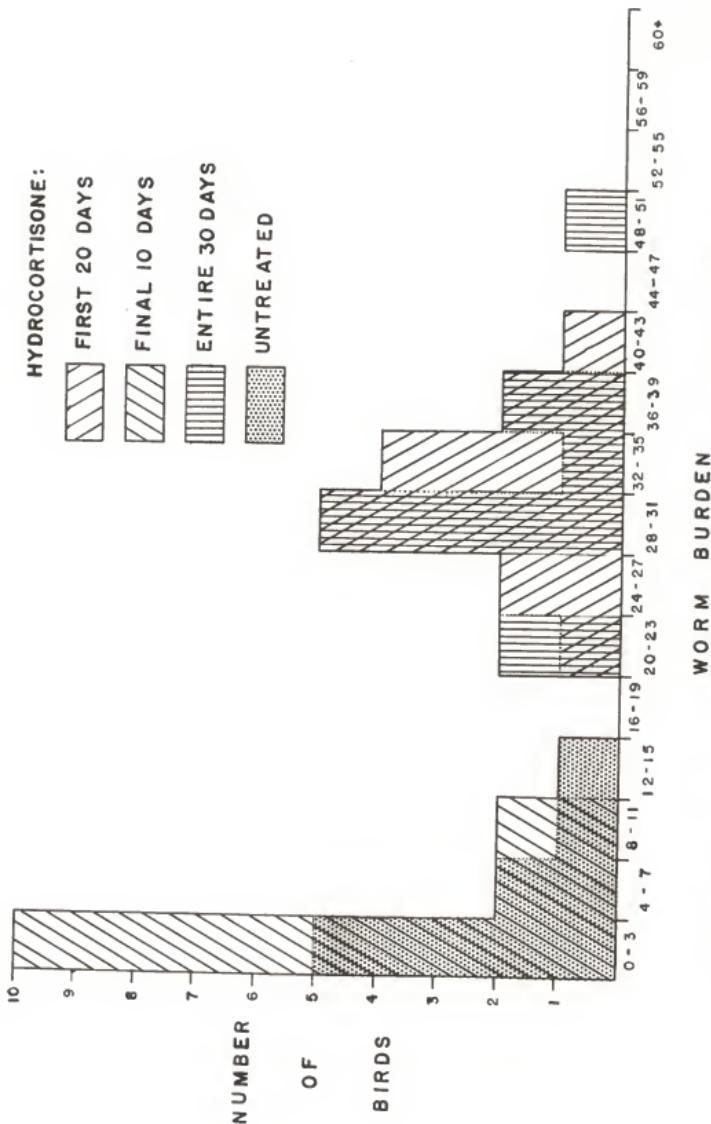


Figure 5. Distribution of worm burden of Leghorn cockerel treated during various periods following infection.

Table 14. Statistical comparison of length of female worms in the different treated groups of Test V

Group	: N	: S	: Ss	: s	: \bar{s}	: \bar{x}
A (hydrocortisone first 20 days post-infection)	316	10027.0	4650.14	3.84	.22	5.29
B (hydrocortisone from 20 to 30 days post-infection)	30	981.0	665.30	4.79	.87	5.45
C (treated controls)	222	6995.0	2939.98	3.65	.24	5.25
D (untreated controls)	23	735.5	321.24	3.24	.68	5.33

Legend:
 for Tables 13 and 14 N = number of worms measured
 S = sum of the length of the worms as measured
 Ss = sum of squares of deviations
 s = $\sqrt{\text{sum of square deviations/observations minus one}}$
 \bar{s} = standard error of mean
 \bar{x} = mean length in centimeters

DISCUSSION

Experimental results of tests in this investigation indicate that injections of hydrocortisone to chickens resulted in treated birds having an increased worm burden of *A. galli*. The increase in worm burden was noted in birds treated with hydrocortisone during the first 20 days post-infection.

Increase in susceptibility to bacterial, protozoan and helminth parasitism in mammals following adrenocorticosteroid treatment has been demonstrated by many workers. Dougherty (1953), Thomas (1953) and Robinson and Smith (1953) described the actions of corticosteroid administration which may

result in increased susceptibility to parasitism as being inhibition of inflammation and suppression of antibody formation. Michael and Whorton (1951), Mogabgab and Thomas (1952), Schwartzman and Aronson (1953), Coker (1955), Weinstein (1955) and Parker (1961) found increased susceptibility to parasitism due to corticosteroid inhibition of the inflammatory response. Fischel (1953), Bjørneboe, et al. (1951) and Briggs (1959) found that corticosteroid suppression of antibody production resulted in increased susceptibility to parasitism.

While no attempt was made in this investigation to determine the mode of action of hydrocortisone in increasing susceptibility of birds to infection of A. galli, it is possible that similar reactions (inhibition of inflammation and antibody formation suppression) take place in the bird as it does in the mammals. As in other vertebrates, corticosteroid treatment of birds results in an involution of the reticuloendothelial system. Glick (1957a), Huble (1958) and Zarrow, Greenman and Peters (1961) demonstrated involution of the bursa of Fabricius and other lymphatic organs following treatment with hydrocortisone or cortisone. Garren and Shaffner (1956) found that when birds were subjected to various stress agents there was involution of the lymphatic tissue. Huble (1955) and Glick (1958) described a relative lymphopenia and an alteration of the polymorphonuclear leucocyte picture following corticosteroid treatment. These actions in the bird would tend to decrease inflammation and to suppress antibody production.

No report was found in the literature of any inflammatory response being involved in the natural defense of the chicken to A. galli. Sadun (1958b, 1949) demonstrated an antibody/antigen reaction in developing resistance. He stated that protection may be furnished by the antibodies working against

the larvae, and that the stage of the parasite during which it burrows into the host's intestinal mucosa may be responsible for stimulating immunity and at the same time, be most vulnerable to the immune effect. The effect of hydrocortisone may be suppression of this antibody production.

The effect of hydrocortisone in increasing worm burden if given during the first 20 days post-infection but not if given after this period suggests that the effect of the hormone may be upon some facet of the host's resistance during the tissue phase of the parasite. Ackert (1931) and Tugwell and Ackert (1948) described the tissue phase of *A. galli* as existing from about the 10th to the 17th day post infection. During the first nine days after hatching the larvae live in the lumen of the small intestine. On about the 10th day, the young larvae bury their anterior ends between intestinal villi and into the glands of Brunner. Sadun (1949) suggested that at this period of the worm's life cycle, its metabolic activity is at its highest due probably to the change in the worm's status from a lumen to a tissue parasite.

A marked difference in weight gain was noted in treated and untreated birds. Hydrocortisone treatment consistently retarded body growth. These results are similar to those found by other workers. Kudzia and Champlon (1953), Dulin (1956), Brown, Brown and Meyer (1958) and Atkinson and Kratzer (1960) all found that corticosteroids inhibited body growth and decreased weight gain in birds at dosages similar or greater to that used in this investigation. Dulin (1955) did not find a decrease in weight gain using 100, 500 or 1000 μ g cortisone in 0.2 ml. saline injections daily but these levels of the hormone are less than those used in these tests. It is generally thought that the failure to gain weight is a result of alterations in carbohydrate and protein metabolism.

It appears that in these tests male worms were longer in treated birds which received hydrocortisone during the first 20 days post-infection than in other birds. Female worms were shorter in the treated birds which received the hydrocortisone during the first 20 days post-infection. The reason for this is unknown. The major effect of hydrocortisone is assumed to be an alteration in the host's resistance rather than in causing a more virulent parasite. Future investigation may determine the effect of hydrocortisone on the physiology of the worm as these results indicate some alteration may occur at least in the growth rate of the worm.

SUMMARY

Injections of hydrocortisone-acetate in saline solution were given to cockerels which had been infected with 100 ± 10 eggs of A. galli when they were 14 days old. Following a period of 30 or 40 days, the birds were sacrificed and the worms recovered from the small intestine. Worm burden of each bird was determined to see the effect of hydrocortisone on worm burden.

Two general test procedures were used: (1) birds were injected with hydrocortisone every three days starting on the day of infection and continuing throughout the entire test period at the end of which the worm burden was compared to untreated control birds worm burden and (2) one group of birds was given the hydrocortisone injections during the first 20 days post-infection and another group of birds was given the hydrocortisone injections during the final 10 days of a 30 day test period. These two groups were compared to two control groups. One control group received hydrocortisone treatment throughout the test period and the other control group remained untreated.

Results of the tests showed:

1. Treatment with hydrocortisone acetate results in an increased worm burden. This increased burden was noted at dosages of 1.25 mg. and 0.625 mg. of hydrocortisone every three days. Birds of both a light breed (White Leghorn) and a heavy breed (Delaware Hampshire) had an increased worm burden when treated with hydrocortisone acetate.
2. Treatment with hydrocortisone during the first 20 days post-infection resulted in an increased worm burden. This increase was not noted if injections of the hormone were started after 20 days post-infection.
3. Male worms were longer and female worms were shorter in birds treated during the first 20 days post infection than worms from birds treated after 20 days post-infection and untreated control birds.
4. Hydrocortisone-treated birds gained less weight than untreated birds. During the period of hydrocortisone treatment average daily weight gain was significantly less than the average daily weight gain of untreated birds.

No attempt was made to determine the mode of action of hydrocortisone in increasing the susceptibility of the chicken to A. galli. It was assumed that some facet of the natural resistance of the chicken to the nematode was altered in such a way as to permit greater numbers of the nematode to survive. The possibility that hydrocortisone may cause some alteration to the host's resistant reaction against the tissue stage of the parasite was indicated by the finding that hydrocortisone produced its effect in increasing worm burden during the first 20 days post-infection. This period of time corresponds to the tissue phase of the parasite.

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Dr. E. H. Herrick, major professor, for his assistance and guidance in experimental work and in preparation of this thesis.

REFERENCES

Abernathy, R.
The effect of cortisone on experimental brucellosis. Jour. Clin. Invest. 30:626-631. 1951.

Ackert, J. E.
The morphology and life history of the fowl nematode Ascaridia galli (Scheider). Parasitology 23:360-379. 1931.

—
Natural resistance to helminthic infections. Jour. Parasit. 28:1-24. 1942.

Ackert, J. E., and L. O. Nolf.
New technique for collecting intestinal roundworms. Science. 70:310-311. 1929.

Ackert, J. E. and J. H. Wimoth.
Resistant and susceptible strains of White Minorca chickens to the nematode, Ascaridia lineata (Schneider). Jour. Parasit. 20:323-324. 1934.

Ackert, J. E., L. L. Eisenbrandt, J. H. Wimoth, B. Glading and I. Bratt.
Comparative resistance of five breeds of chickens to the nematode Ascaridia lineata (Schneider). Jour. Agr. Res. 50:607-624. 1935.

Ackert, J. E., S. A. Edgar, and L. P. Frick.
Duodenal goblet cells and age resistance to intestinal parasitism. Third Inter. Cong. Microsc. Organ., Proc. 481-482. 1939.

Ackert, J. E. and S. A. Edgar.
Intestinal goblet cells and age resistance to parasitism. Jour. Parasit. 26 (Suppl.):14-15. 1940.

Ackert, J. E. and L. W. Dewhirst.
Resistance of fowls to parasitism affected by female sex hormone. Jour. Parasit. 36 (Suppl.):16. 1950.

Atkinson, R. L. and F. H. Kratzer.
Influence of cortisone, Liver L and dienstrol diacetate on the body and organ weight of male chicks. Poult. Sci. 39:638-645. 1960.

Baughn, C. O.
The effect of adrenalectomy on natural and acquired resistance of mice to Trichinella spiralis. Jour. Elisha Mitchell Scientific Society 68:207-221. 1952.

Berlin, B. S., C. Johnson, W. D. Hawk and A. G. Lawrence.
The occurrence of bacteremia and death in cortisone-treated mice. Jour. Lab. and Clin. Med. 40:82-89. 1952.

Bezubik, B.

Effect of cortisone on the susceptibility of hamsters and guinea pigs to the sheep and rabbit strains of Strongyloides papillosum. Jour. Parasit. 46(Suppl.):30-41. 1960.

Bjørneboe, M., E. E. Fischel and H. C. Stoerk.

The effect of cortisone and adrenocorticotropic hormone on the concentration of circulating antibody. Jour. Exp. Med. 93:37-40. 1951.

Briggs, N. T.

The effect of cortisone on natural resistance and acquired responses of the white rat to infection with Litomosoides carinii. Jour. Parasit. 45(Suppl.):37. 1959.

Brown, K. I., D. J. Brown and R. K. Meyer.

Effect of surgical trauma, ACTH and adrenal cortical hormones on electrolytes, water balance and gluconeogenesis in male chickens. Amer. Jour. Physiol. 192:43-50. 1958.

Coker, C. M.

Effects of cortisone on Trichinella spiralis infections in non-immunized mice. Jour. Parasit. 41:498-504. 1955.

Some effects of cortisone in mice with acquired immunity to Trichinella spiralis. Jour. Infect. Diseases. 98:39-44. 1956a.

Cellular factors in acquired immunity to Trichinella spiralis, as indicated by cortisone treatment of mice. Jour. Infect. Diseases. 98:187-197. 1956b.

Effects of cortisone on cellular inflammation in the musculature of mice given one infection with Trichinella spiralis. Jour. Parasit. 42:479-484. 1956c.

Effect of cortisone on natural immunity to Schistosoma mansoni in mice. Proc. Soc. Exp. Biol. and Med. 96:1-3. 1957.

Cross, J. H.

The natural resistance of the white rat to Nematospirodes dubius and the effect of cortisone on this resistance. Jour. Parasit. 46:175-185. 1960.

Davis, D. E. and J. J. Christian.

Relation of adrenal weight to social rank in mice. Proc. Soc. Exp. Biol. and Med. 99:269-272. 1958.

Davis, D. E. and C. P. Read.
Effect of behavior on development of resistance in trichinosis. Proc. Soc. Exp. Biol. and Med. 99:269-272. 1958.

Dougherty, T. F.
Some observations on mechanism of corticosteroid action on inflammation and immunological processes. Ann. N. Y. Acad. Sci. 56:748-755. 1953.

Dougherty, T. F., H. E. Brown and D. L. Berliner.
Metabolism of hydrocortisone during inflammation. Endocrinol. 62:255-262. 1958.

Dulin, W. E.
The effects of cortisol on the White Leghorn cockerel and capon. Poult. Sci. 34:73-77. 1955.

Eisenbrandt, L. L. and J. E. Ackert.
On the resistance of the chicken to the nematode, Ascaridia perspicillatum. Amer. Jour. Hyg. 32:246-253. 1940.

Fischel, E. E.
Adrenal hormones and the development of antibody and hypersensitivity. In "Effect of ACTH and Cortisol upon Infection and Resistance" (G. Schwartzman, ed.) pp. 88-96. Columbia University Press, New York. 1953.

Fischel, E. E., H. C. Stoerk and M. Bjørneboe.
Failure of cortisol to affect rate of disappearance of antibody protein. Proc. Soc. Exp. Biol. and Med. 77:111-123. 1951.

Frick, L. P. and J. E. Ackert.
Further studies on duodenal mucus as a factor in age resistance of chickens to parasitism. Jour. Parasit. 34:192-206. 1948.

Garren, A. W. and C. S. Shaffner.
How the period of exposure to different stress stimuli affects the endocrine and lymphphatic gland weights of young chickens. Poult. Sci. 35:266-272. 1956.

Glick, B.
Experimental modification of the growth of the bursa of Fabricius. Poult. Sci. 36:13-23. 1957.

The effect of cortisol acetate on the leukocytes of young chickens. Poult. Sci. 37:1446-1452. 1958.

Hansen, M. F., M. G. Norris and J. E. Ackert
The influence of an all plant protein diet supplemented with aureomycin
a and Vitamin B₁₂ on the resistance of chicks to Ascaridia galli,
(Schrank). Poult. Sci. 32:612-637. 1953.

Hansen, M. F., L. J. Olson and J. E. Ackert.
Improved techniques for culturing and administering ascarid eggs to
experimental chicks. Exper. Parasit. 3:464-473. 1954.

Hansen, M. F., C. J. Terhaar and D. S. Turner.
Importance of the egg shell of Ascaridia galli to the infectivity of its
larvae. Jour. Parasit. 42:122-125. 1956.

Herrick, C. A.
Studies on the resistance of chickens to the nematode Ascaridia perspicillum. Amer. Jour. Hyg. 8:125-127. 1926.

Huble, J.
Haematological changes in cockerels after ACTH and cortisone-acetate
treatment. Poult. Sci. 24:1357-1359. 1955.

Effects of hormones on endocrine and lymphoepithelial glands in young
fowl. Poult. Sci. 37:297-300. 1958.

Jones, F. E., M. L. Melton, M. N. Lunde, D. E. Eyles and L. Jacobs.
Experimental toxoplasmosis in chickens. Jour. Parasit. 45:31-37. 1959.

Kalter, S. S., H. J. Smolin, J. M. McElhaney and J. Tepperman.
Endocrines and their relation in influenza virus infection. Jour. Exp.
Med. 93:529-538. 1951.

Kass, E. H., S. H. Ingbar, M. M. Lundgran and M. Finland.
The effect of ACTH and cortisone on pneumococcal and influenza viral
infections in white mice. Jour. Lab. and Clin. Med. 37:780-788. 1951.

Kilborne, E. D. and F. L. Horsfall, Jr.
Increased virus in eggs injected with cortisone. Proc. Soc. Exp. Biol.
and Med. 76:116-120. 1951a.

Lethal Infection with Coxsackie virus of adult mice given cortisone.
Proc. Soc. Exp. Biol. and Med. 77:135-138. 1951b.

Kudzia, J. J. and L. R. Champion.
Investigations concerning the effects of cortisone in the domestic fowl.
Poult. Sci. 32:476-481. 1953.

Larson, I. W.
Action of certain anthelmintics on Ascaridia galli (Schrank, 1988) and
on Heterakis gallinarum (Schrank, 1788). Unpublished Master's thesis.
Kansas State College. 1957.

Lord, R. A.
Studies on the use of cortisone and ACTH in trichinosis. Amer. Jour. Trop. Med. and Hyg. 7:611-617. 1958.

Markell, E. K., and W. P. Lewis.
Effect of cortisone treatment on immunity to subsequent reinfection with *Trichinella* in the rat. Amer. Jour. Trop. Med. and Hyg. 6:553-561. 1957.

McGregor, J. K.
Observations on the therapeutic value of adrenocorticotropic hormone in clinical enterohepatitis (blackhead) in turkeys. Can. Jour. Comp. Med. 18:332-334. 1954.

Michael, M. Jr. and C. M. Whorton.
Delay of the early inflammatory response by cortisone. Proc. Soc. Exp. Biol. and Med. 76:754-756. 1951.

Mogabgab, W. J. and L. Thomas.
The effects of cortisone on bacterial infection. Jour. Lab. and Clin. Med. 39:271-289. 1952.

Nobel, G. A.
Stress and parasitism. I. A preliminary investigation of the effects of stress on ground squirrels and their parasites. Exper. Parasit. II: 63-67. 1961.

Parker, J. C.
Effect of cortisone on the resistance of the guinea pig to infection with the rat nematode, *Nippostrongylus brasiliensis*. Exper. Parasit. II: 380-390. 1961.

Pullin, J. W.
Observations on the use of cortisone in experimental enterohepatitis in turkeys. Can. Jour. Comp. Med. 19:67-68. 1955.

Redmond, W. B.
Influence of cortisone on natural course of malaria in the pigeon. Proc. Soc. Exp. Biol. and Med. 79:258-261. 1952.

Riedel, B. B., and J. E. Ackert.
The resistance of chickens to ascarids as affected by protein supplements of soybean oil meal and skim milk. Poult. Sci. 29:437-443. 1950.

Ritterson, A. L.
Innate resistance of species of hamsters to *Trichinella* and its reversal by cortisone. Jour. Infect. Diseases. 105:253-266. 1959.

Robinson, H. J. and A. L. Smith.
The effect of adrenal cortical hormones on experimental infection. Ann. N. Y. Acad. Sci. 56:757-764. 1953.

Roman, E.

Spécificité parasitaire de Strongyloides ratti, du surmulot. Effets de la cortisone sur l'infestation d'autres rongeurs par ce nematode. Annales de parasitologie humaine et comparée. 31:552-571. 1956. Original not seen, Abstract in Biological Abstracts, Sec. C. 32(8) No. 28688. 1958.

Sadun, E. H.

Relation of the gonadal hormone to the natural resistance of chickens and to the growth of the nematode, A. galli. Jour. Parasit. 34(Suppl.): 18. 1948a.

Resistance induced in chickens by infections with the nematode Ascaridia galli. Amer. Jour. Hyg. 47:282-289. 1948b.

The antibody basis of immunity in chickens to the nematode Ascaridia galli. Amer. Jour. Hyg. 49:101-116. 1949.

Sadun, E. H., E. K. Kelth, M. J. Pankey and J. R. Totter.

The influence of dietary pteroylglutamic acid and A.P.A. liver extract on survival and growth of the nematode Ascaridia galli in chickens fed purified and natural diets. Amer. Jour. Hyg. 51:274-291. 1950.

Selye, H.

The general adaptation syndrome and the diseases of adaptation. Jour. Clin. Endocrinol. 6:117-230. 1946.

The general adaptation syndrome and the diseases of adaptation. Jour. of Allergy 17:231-247 and 258-298. 1946.

Stress, Acta Endocrinologica, Montreal, Canada. 822pp. 1950.

Perspectives in stress research. In "Perspectives in Biology and Medicine" 2:403-416. 1959.

Shwartzman, G., and S. J. Aronson.

Alteration of experimental poliomyelitis by means of cortisone with reference to other viruses. In "Effect of ACTH and Cortisone upon Infection and Resistance" (G. Shwartzman, ed.) pp. 176-204. Columbia Press, New York. 1953.

Stoner, R. D. and J. R. Godwin.

The effects of ACTH and cortisone upon susceptibility to Trichinosis in mice. Amer. Jour. Path. 29:943-950. 1953.

Stoner, R. D. and J. R. Godwin.

The effects of adrenocorticotropic hormone and cortisone upon acquired immunity to trichinosis in mice. Amer. Jour. Path. 30:913-918. 1954.

Thomas, L.

Effect of cortisone and adrenocorticotropic hormone on infection. Ann. Rev. Med. 3:1-13. 1952.

Cortisone and infection. Ann. N. Y. Acad. Sci. 56:799-814. 1953.

Todd, A. C.

Thyroid condition of chickens and the development of parasitic nematodes. Jour. Parasit. 35:255-260. 1949.

Todd, A. C. and D. H. Crowdus.

Methyl testosterone in the diet of chicks and growth of the nematode, Ascaridia galli. Jour. Parasit. 37:322-326. 1951.

Tugwell, R. L. and J. E. Ackert.

On the tissue phase of the life cycle of the fowl nematode, Ascaridia galli (Schrank). Jour. Parasit. 38:277-288. 1952.

Weinman, C. J. and G. W. Hunter

Studies on schistosomiasis. XIV. Effects of cortisone upon the Schistosoma mansoni burden in mice. Jour. Parasit. 45(Suppl.):16-17. 1959.

Weinstein, P.

The effect of cortisone on the immune response of the white rat to Nippostrongylus muris. Amer. Jour. Trop. Med. and Hyg. 4:61-74. 1955.

Wolf, A., E. A. Kabat, A. E. Bezer and J. R. Fonseca.

The effect of cortisone in activating latent trypanosomiasis in rhesus monkeys. In "Effect of ACTH and Cortisone upon Infection and Resistance", (G. Schwartzman, ed.) pp.122-139. Columbia Press, New York, 1953.

Zarrow, M. X., D. L. Greenman, and L. E. Peters.

Inhibition of the bursa of Fabricius and the stilbesterol-stimulated oviduct of the domestic chick. Poult. Soc. 40:87-93. 1961.

**INFLUENCE OF HYDROCORTISONE ON THE SUSCEPTIBILITY OF CHICKENS
TO THE NEMATODE, ASCARIDIA GALLI (SCHRANK, 1788)**

by

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

**KANSAS STATE UNIVERSITY
Manhattan, Kansas**

1962

The susceptibility of chickens to the large intestinal roundworm, Ascaridia galli, is dependent upon several factors. Previous investigators have shown that age, breed and nutritional status of the bird influence development of worms in the chicken's intestine. Because there is a wide variation within a group of birds receiving the same number of worm eggs at infection, hormonal factors have been implicated in the natural resistance of the chicken to this parasite. The purpose of this investigation was to test the influence of the adrenal-cortical hormone, hydrocortisone, on the susceptibility of chickens to the nematode, A. galli.

A review of the literature showed many reports of increased susceptibility following injections of glucocorticoids, principally cortisone. The action of the glucocorticoids in decreasing resistance in these reports followed two main pathways: (1) decreasing of an inflammatory response which would normally inhibit the parasite's development and (2) suppressing the production of an antibody. Either or both may play a part in the host's natural resistance to a parasite.

In this investigation a total of 237 male White Leghorn and 50 male Delaware Hampshire chickens was used. In each of the five tests birds were divided into treated and control groups. When the chicks were 14 days old, 100 ± 10 A. galli eggs were given per os. to all birds. Treatment of the treated group with hydrocortisone began on the day of infection in some cases and on the 20th day post-infection in others. Birds were kept for a period of 30 or 40 days following infection at which time they were sacrificed and the worm burden for each bird was determined. During the test period weight records were kept for each bird. Worms from two tests were sexed and measured.

A significantly greater worm burden was found in the treated birds. The

Increase in worm burden followed injections of either 1.25 mg. or 0.625 mg. of hydrocortisone given intramuscularly every three days. The increase was noted in both the White Leghorn (a light breed) and the Delaware Hampshire (a heavy breed) chickens.

To determine more exactly when hydrocortisone injections were instrumental in lowering resistance to the parasite, the period of hydrocortisone injection was varied. Chickens treated during the first 20 days post-infection had a significantly greater worm burden than control birds. Those birds treated only following the 20th day post-infection did not have a significantly greater worm burden.

No attempt was made in this investigation to determine the mode of action of hydrocortisone in increasing worm burden. There is a possibility that the hydrocortisone may interfere with the production of an antibody during the tissue phase of A. galli.

Birds receiving hydrocortisone gained significantly less weight than untreated control birds during the test period in all cases.

Statistical analyses showed that male worms were longer and female worms were shorter in birds treated with hydrocortisone during the first 20 days post-infection.